Chromatography of a Popular Consumer Beverage

PURPOSE

- Use paper chromatography to separate the food dyes in a popular colored beverage.
- Determine the identities of the dyes in food coloring, using the known dyes in the popular consumer beverage as standards.

INTRODUCTION

A **mixture** is a physical phenomenon, a combination of two or several substances. Because each component of a mixture retains its chemical identity and characteristics, a mixture can be separated into its chemical components by exploiting physical differences. For example, suppose that you were given the task of separating a mixture of bowling balls and Ping-Pong balls. This mixture might be sifted with a volleyball net to exploit the physical characteristic of *size*: the bowling balls being held within the net, while the smaller Ping-Pong balls passed through the openings. Or, you might exploit the physical characteristic of *density* by pouring your mixture of balls into a swimming pool and noting that the bowling balls sink to the bottom of the pool, while the less dense Ping-Pong balls stay at the surface. You might also exploit the physical characteristic of *color* by separating the white Ping-Pong balls from the darker bowling balls. In each case, you have identified a physical difference between or among the components of a mixture and used that characteristic to sort those components.

One laboratory method of separation is called **chromatography**, from the Greek *chroma*, meaning "color," and *graphein*, meaning "to write." The method depends on the tendencies of different components of a mixture to have varying degrees of adsorption onto solid surfaces. In general, chromatographic methods work by using a combination of two phases of matter, commonly one in the solid state and one in the liquid state. The solid represents the **stationary phase**, while the liquid (or, sometimes, a gas) represents the **mobile phase**. Typically a liquid mixture of components is passed over a solid, and the difference in relative attraction of each liquid for the solid can be used to separate those components physically. A component with little attraction for the solid will pass quickly by the surface of the solid, whereas a substance with greater attraction for the surface of the solid will linger longer. There are a variety of types of chromatography, but all exploit the characteristic of preferential adsorption onto a solid surface. Methods include thin-layer chromatography and gas chromatography.

One popular type of chromatography is **paper chromatography**. A strip of filter or other porous paper is used as the stationary phase. A concentrated spot of the mixture to be separated is dotted onto the lower end of the paper strip. The end of the paper strip is then dipped into the mobile phase, which is a mixture of liquid solvents. The liquid phase gets wicked up the strip of paper and, in so doing, passes by the dot of the mixture, attracting its components and moving the dot up the paper. Different components of the mixture are carried different distances up the paper. Components with little attraction for the solid paper are carried farther up than those components that adsorb better onto the paper. The liquid solvent mixture can be altered to change its rate of movement up the solid phase.

To measure the differences in adsorption onto a solid surface, you may determine a value called the R_f, or ratio of fronts. The R_f is a ratio that compares the distance traveled by a component of the mixture to the distance traveled by the solvent:

$$R_f(component) = \frac{D_{spot\ front}}{D_{solvent\ front}}$$

where $D_{spot\ front}$ indicates the distance traveled by the front of the spot of the component of the mixture being considered and $D_{solvent\ front}$ represents the farthest distance traveled by the solvent. Note that R_f values are characteristic of a solute-solvent combination under specified conditions. Changing the temperature of the system, for example, or the material of the solid phase, may change the value of the R_f.

You may have used paper chromatography in your biology class to separate chlorophyll into its components, chlorophyll a and chlorophyll b.

Procedure Preview In this experiment you will take a concentrated solution made from a powdered beverage containing known food dyes, and use these dyes as standards in a chromatography experiment to determine the identity of the dyes in food coloring. The ingredients in food colorings are normally listed on the outside of the box containing a variety of different colors; however, this list does not break down the specific dyes in each individual food coloring. But each powdered beverage does show the specific list of food colors it contains, so you can separate them using paper chromatography and a solvent system, or a developing solution, containing a mixture of aqueous ammonia, ethanol, and isopropanol, and then use the results as standards. The identity of the food dyes in the food coloring can be determined by comparing the distance that each individual food dye in the food coloring traveled on the paper to the distance that the standard food dyes in the powdered beverage solution moved on the paper.

Pre-Lab Questions

1. From your teacher, obtain a list of food dyes in the powdered beverage and in the food coloring samples you will use in your experiment. Find their chemical structures in the *Merck Index* or other chemical database. Draw the structures of each food dye.

Drink Flavor	Food Dyes
Cherry	Red 40, Blue 1
Grape	Red 40, Blue 1
Lemon Lime	Yellow 5, Blue 1
Orange	Yellow 5, Red 40

2. Read through all the steps of the Procedures below. Why do you think the development chamber must be sealed during this experiment? How fast would the experiment proceed if a student forgot to seal the development chamber?

3. Why do we use a pencil to mark chromatography paper, rather than a ballpoint or felt-tipped pen? What would happen if we used one of these pens instead?

MATERIALS

- samples of food colorings
- powdered beverage mixes (each containing known food dyes)
- scoopulas/spoons
- chromatography paper (alternatively, Whatman Number 1 filter paper)
- ruler, pencil, & stapler
- toothpicks
- 24-well microplate
- developing solution (consisting of 50 mL household ammonia, 25 mL 70% isopropanol, 25 mL 91% ethanol)
- 600-mL beaker (as developing chamber)
- plastic wrap/parafilm (to seal developing chamber)

PROCEDURE

I. Preparing solutions of powdered beverage and the chromatography paper

Step A Carefully add enough powdered beverage mix to specific wells in the microplate, being sure not to contaminate surrounding wells with other samples. Record the well to which each sample is added. Use a clean and dry scoopula or spoon for each sample.

Step B Add enough hot water to fill each well containing a sample about two-thirds full, then use a new toothpick to mix and dissolve each sample in the hot water.

Step C Cut chromatography paper into a 10-cm × 20-cm rectangle. The 20-cm side is the horizontal axis.

Step D Use a pencil to lightly draw a straight line 1 cm from the bottom of the rectangle. Draw two other lines, each 1 cm from the rectangle sides.

Step E Lightly mark the bottom line every 2 cm; these marks are where you will spot the paper with each sample. Lightly number each mark underneath the horizontal line with the pencil.

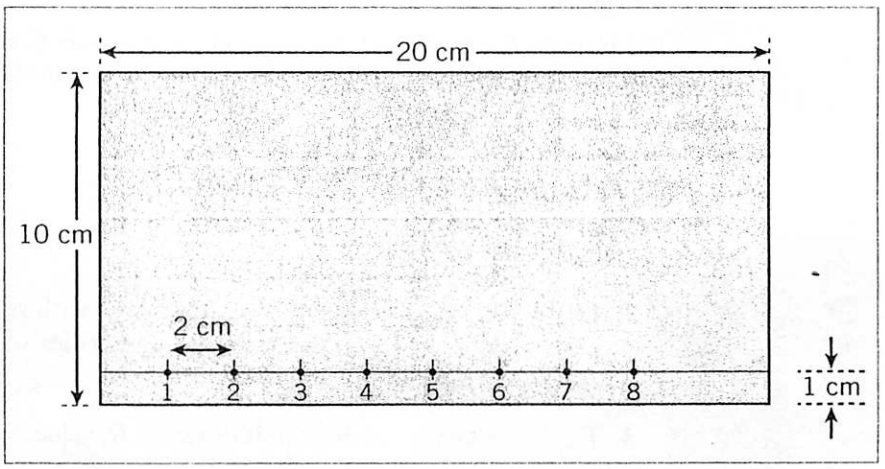

Figure 15.1

PROCEDURE *(continued)*

II. Spotting chromatography paper with samples and developing the sample

Step A Using a new toothpick for each sample, carefully transfer a drop of each beverage to a different numbered spot on the paper. Be sure that no solid gets transferred in the drop.

Step B Record the number for each sample in your notebook.

Step C Add a few drops of each food coloring to its own microwell, and transfer a drop to the chromatography paper using a new toothpick. Record the number for each food-coloring sample in your lab notebook.

Step D Form a roll with the paper, so that the samples are at the bottom and the spots on the inside.

Step E Carefully staple the roll at the top and bottom, leaving a small gap between the edges of the paper. First make sure the sample spots have dried.

Step F Pour the developing solution into the bottom of the developing chamber so that liquid level is ***below*** the spotted samples. Be sure not to disturb the dye spots.

Step G Cover developing chamber and watch the solvent move up the paper. Be sure to monitor solvent progress, as you will need to remove the paper from the chamber when the solvent has moved to 1–2 cm from the top of the paper.

Step H When solvent reaches this level, remove the paper from the chamber, open the paper roll, and quickly trace the solvent front with a pencil.

Step I Let the paper dry, being sure to notice if the solvent front has moved any farther up the paper. If it has moved as the paper dries, retrace the solvent front. When the paper is dried, circle all colors from the separated dyes with a pencil, and mark the top of each dye with a pencil. Record the relative color shades and intensities of each color in your lab notebook.

Step J Use a ruler to measure the distance between the starting point of the sample and this line at the top of the dye spot, as well as the distance from the starting point to the solvent front.

Calculations

Calculate the R_f value for each dye sample of powdered mix. Compare these R_f values, which are your standards, to those of the unknown dyes in the food-coloring samples.

Post Lab Questions:

1. Construct a data table.

2. Use the R_f values you have calculated, along with your detailed color observations, to try to determine the identities of the dyes in the food-coloring samples.

3. Which dye is most attracted to the solvent? Which dye is most attracted to the paper? Which dye is the least polar, which is the most polar?

4. Explain why the colored dyes exhibit different R_f values.

5. In modern chemistry, chemists use principles of green chemistry to evaluate solvents that are used in a chemical process for their level of toxicity to humans and the environment. Solvents are also evaluated in terms of their life cycle or how long the molecule remains in the environment and if the breaks down to become more benign or more toxic. The overall focus of green chemistry is to be more efficient in chemical production, producing less waste, using fewer toxic molecules, and producing waste that biodegrades and does not pose a risk to the environment.

Compare the "greenness" of hexane to that of 2-propanol and water. Explain your answer.

6. Draw a picture of how the chromatography worked. Explain your picture using the following terms: stationary phase, mobile phase, and intermolecular forces.